BEHAVIORAL AND INTEGUMENTARY CHANGES ASSOCIATED WITH INDUCED METAMORPHOSIS IN DIEMICTYLUS [1]

WILLIAM C. GRANT, JR. AND GEORGE COOPER, IV [2]

Department of Biology, Williams College, Williamstown, Massachusetts

After metamorphosis the newt, *Diemictylus viridescens,* passes into a terrestrial (eft) phase for two or three years, following which the animal migrates to water and assumes adult characteristics. This return to the natal environment has been termed a "second metamorphosis" by Wald (1960). Grant and Grant (1958), following the suggestions of Chadwick (1941), have shown that migration to water and associated skin changes from the granulated to smooth condition can be initiated in the hypophysectomized eft with 1.4 μg. of highly purified prolactin (Li). Similar responses have been produced in efts by Masur (1962) following pituitary autotransplantation, which suggests an inhibitory control exerted by the hypothalamus over the release of water-drive principles from the adenohypophysis. Waterman and Grant (1961) reported 48-hour thyroidal uptake of I^{131} to be 15.8% of original dose in efts as compared to 1.8% in adults. Prolactin-treated efts showed a shift towards adult values with lowered uptakes at 6.9%.

According to Etkin (1964) primary metamorphosis in urodeles can be compared with anuran metamorphic climax, as it also involves the growth of glands and pigment and the degeneration of caudal fins and external gills. The concept that thyroxin is associated with tail fin degeneration has recently been reinforced by Gross (1964), who found that collagenolytic activity of tail fin tissue from thyroxin-treated tadpoles increased significantly over that of controls. The process of tail resorption is complicated, involving a laying down of new collagen fibers in basement membranes and lysis of older fibers by collagenase released from epidermal cells. Gradual dissolution of basement membranes is the prime factor in tail regression. Kaltenbach (1953) has shown the effect of thyroxin in producing localized metamorphosis in *Rana pipiens* tadpoles, and Kaltenbach and Clark (1965) have demonstrated the direct effect of embedded pellets of thyroxin and thyroxin analogues on the skin of *Diemictylus viridescens.*

Activity of the pituitary-thyroid axis during primary metamorphosis is too well known to be reviewed here, but mention of some pituitary-hypothalamic relationships is essential. Transplantation of adenohypophysial primordia in *Rana pipiens* tadpoles reported by Etkin (1938), and Etkin and Lehrer (1960), and sectioning of the infundibular stalk in *Ambystoma* larvae by Etkin and Sussman (1961) indicate that some continuity between the hypothalamus and hypophysis is necessary to sustain TSH release during metamorphic climax. As larvae operated upon in this manner continue to grow, the possibility exists that amphibian prolactin released after removal of hypothalamic inhibition acts as a growth factor. Recently,

[1] Supported by Grants AM 01786-07 and HD 01164-08 from the NIH and NSF Grant GE-1099.

[2] Present address: Cornell University Medical College, New York, New York.

the important paper of Berman *et al.* (1964) has substantiated the role of prolactin as a larval growth factor following intraperitoneal injection of highly purified prolactin and bovine STH into *Rana catesbeiana* tadpoles.

Other factors occurring during primary metamorphosis have not been studied in detail. For example, there is still considerable controversy over the relationship of thyroxin to metabolic rates; no increase in rate was reported during metamorphosis in *Rana grylio* by Lewis and Frieden (1959), while Barch (1953) found increased oxygen consumption in isolated skin from metamorphosing larvae and in skin from the area of thyroxin-pellet implants. Kaye (1961) showed an increase in thyroid activity during metamorphosis in *Rana pipiens* tadpoles in which 43% I^{131} uptake occurred at the end of the premetamorphic period.

Gorbman (1964) suggested that molting, an important characteristic of amphibian skin not restricted to periods of metamorphosis, should be considered as two separate processes, the first involving proliferation and cornification of the epidermis and the second sloughing of epidermal structures. This concept is validated by the fact that experimental procedures which inhibit sloughing do not necessarily affect proliferation of the epidermis. The production of local molts in the area of thyroxin-pellet implants in thyroidectomized animals by Clark and Kaltenbach (1961) and the extended series of experiments of Adams and her co-workers (Adams, 1932; Adams and Grierson, 1932; Adams and Richards, 1929; Adams *et al.*, 1932, etc.) would indicate that molting activity is conditioned by the direct action on the skin by thyroxin whose production is regulated by a pituitary-thyroid control mechanism. That the molting process may be more complex is evidenced by the work of Chadwick and Jackson (1948) and Grant and Grant (1958) on *Diemictylus (Triturus) viridescens*, where prolactin increased epidermal mitotic rates in normal animals and elicited irregular sloughing following hypophysectomy. Both the thyroid and adrenal cortex may activate slough preparation and sloughing to varying degrees in *Ambystoma* and *Bufo*, according to Jørgensen and Larsen (1960, 1961), who also consider that frequency of molting is maintained by levels rather than cycles of thyroid activity.

The present investigation was undertaken in order to explore further the control exerted by both thyroxin and prolactin on primary and secondary metamorphosis in *Diemictylus*, with special attention directed towards related epidermal changes.

Materials and Methods

The efts and adults of *Diemictylus* used in these studies were obtained freshly caught from a supplier, while the larvae were collected locally during the summer. In the first series of experiments four groups of 20 adult animals received injections every other day according to the following protocol: group A, 0.1 ml. amphibian Ringer's; groups B and b, 5 μg. of L thyroxin sodium in 0.1 ml. of amphibian Ringer's until the end of four days when it was increased to 20 μg. per injection; group C, 100 μg. of ovine prolactin (NIH) in Ringer's solution. A fifth group of 20 animals, D, was placed in a terrestrial environment on moss (mostly *Polytrichum*). Each of groups A, B, b, and C was placed in a "choice" situation which consisted of plastic containers (6 × 10 in.) separated into equal areas of land and water by plastic partitions ¾ in. high. This allowed the animals to move freely from one area to another. Each container held five test animals and daily observations were made to determine the distribution of animals in either of the two environments. On the eleventh day of this experiment groups B and C were divided into three groups each: one which continued to receive the original treatment, one which received no further treatment, and one which received the reciprocal of its original injection (*i.e.*, thyroxin-treated animals received prolactin and *vice versa*). Half of group D was now offered a land-water choice situation and group A continued as controls. In another experiment each of 36 efts was injected with 100 μg. of prolactin in order to study skin changes associated with induced water migration.

Histological sections of skin cut at 7 μ were prepared from the mid-dorsal and lower jaw region of animals in each experimental group and stained with either Harris' hematoxylin-eosin or Masson's trichrome stain. Photomicrographs of slides were made with a Unitron MiC3-369 microscope.

In the second series of experiments larvae of *Diemictylus* were divided into pre-melanic and melanic groups, the latter representing the darkened phase occurring just prior to primary metamorphosis. Animals were maintained in plastic cups in which a stender dish covered with moist filter paper provided a land environment. Pre-melanic animals (0.12 gm. average weight) were divided into two groups; one received intraperitoneal injections of 0.01 ml. Ringer's solution twice a week by glass capillary needle and another 10 μg. prolactin at the same volume. Another group was placed in sterile pond water containing 1 μg./ml. thyroxin. Three groups of melanic stage animals received similar treatment. Periodic observations were made of metamorphic events on the staged scale of pre-melanic, melanic, gill loss, and land migration which terminated primary metamorphosis. These stages correspond to the phases of primary metamorphosis reported by Noble (1929) for *Diemictylus*.

Groups of adults which were shifted to a land environment were injected intraperitoneally with 1 microcurie of radiodide after ten days on land. Counts of thyroids were made at 24, 48, 72 and 96 hours on a scintillation counter, and uptakes expressed as a per cent of the injected dose of I^{131}. Uptakes of a control group of aquatic adults were taken at 24 and 72 hours. All experiments reported above were conducted at approximately $22 \pm 1°$ C.

Other investigations were made to determine the direct effect of thyroxin and prolactin on newt skin in tissue culture. Adult newts were anesthetized in 0.1% MS222, immersed briefly in a 5×10^{-4}% aqueous merthiolate-Ringer's solution and placed in a sterile cabinet. Tissue sections 1 mm. square were excised from the mid-dorsal region of the animals under a mercury vapor lamp (GE #G8T5) and placed for an hour in deep-well slides containing the antibiotic salt solution of Fimian (1959). Three tissue fragments were placed in each of a number of Carrel D-50 flasks on a 1-ml. coagulum containing chick plasma (Difco), chick embryo extract (Difco EE-100) and antibiotic solution in a 3:1:5 ratio. Tissue fragments were then covered with 2 ml. of antibiotic solution (0.6 gm./liter of penicillin and streptomycin) which was changed every three days. Control tissues were incubated in saline solution while experimental tissues were treated with solutions of either 0.2 mg./ml. thyroxin or 1 mg./ml. prolactin (NIH and highly purified samples by Li). Tissue fragments were removed periodically for histological analysis.

Results

Water-land migration in adults (Fig. 1)

Thyroxin-treated animals began to emerge from the water on the sixth day and by the eleventh all members of group b and 80% of group B were terrestrial. A large percentage of animals in group B which continued to receive thyroxin treat-

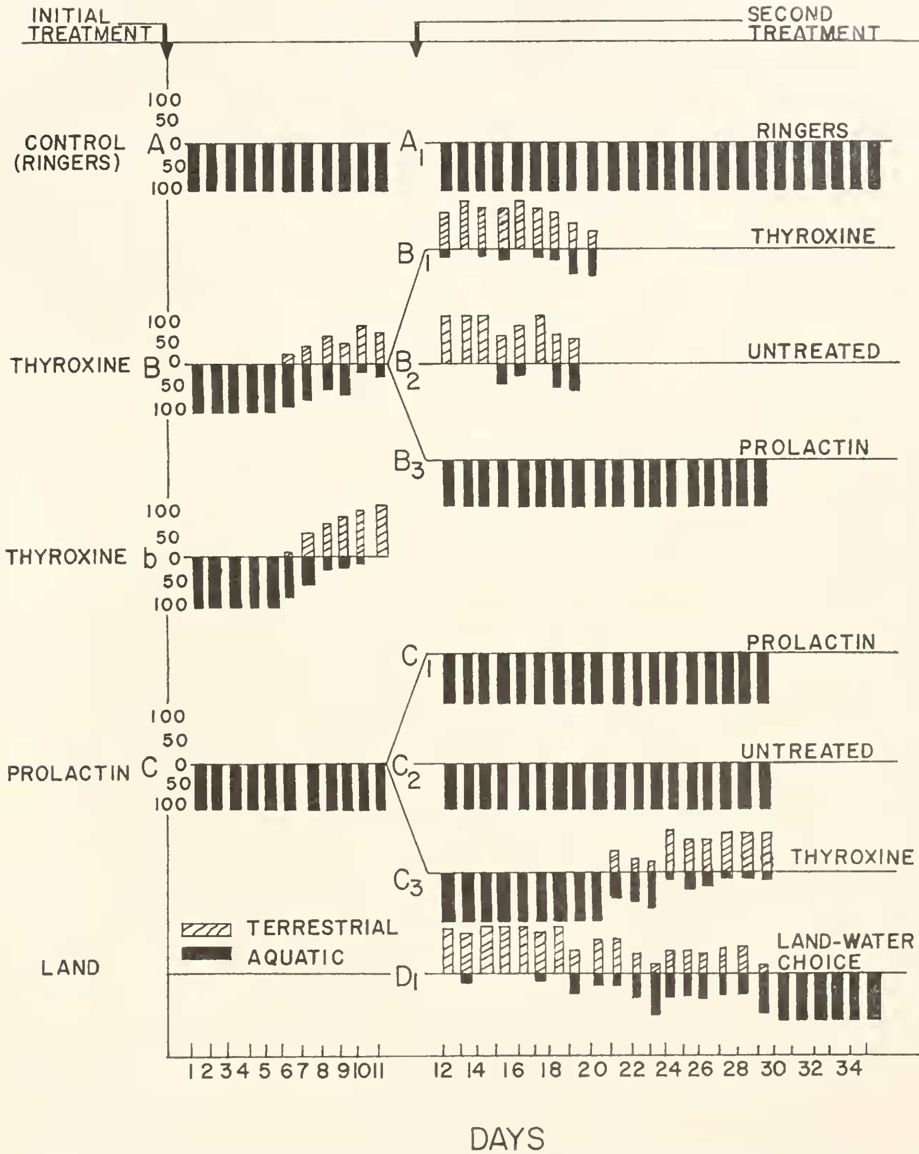

FIGURE 1. Per cent distribution of adults of *Diemictylus* in choice cages with aquatic or terrestrial environments, following treatment with thyroxin and prolactin.

ment or which were left untreated after the eleventh day continued on land although some returned to water towards the termination of the experiment. Group B animals which received prolactin on the twelfth day became aquatic within a day and remained so until termination of the experiment.

No members of the control group A or the prolactin-treated group C were ever observed on land during the entire course of the experiment. In those group C animals treated with thyroxin after cessation of prolactin injections a land-drive occurred approximately 10 days after the initiation of thyroxin injections, as compared to the six-day latent period for group B animals. Animals were recorded

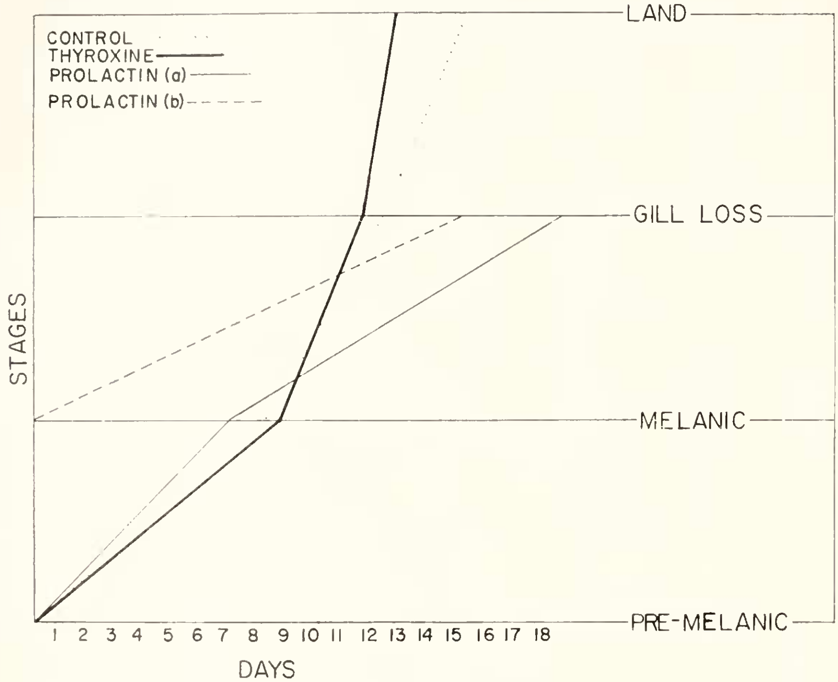

FIGURE 2. Average number of days for larvae of *Diemictylus* to pass through pre-melanic, melanic, and gill loss stages following injection of thyroxin or prolactin.

on land an average of 88% of the time during the last three days of the experiment which began 15 days after the first treatment with thyroxin.

Group D animals which had been land-conditioned on moistened *Polytrichum* showed a gradual shift to water which was completed by the eleventh day when they were presented with a choice of environments.

Effects of thyroxin and prolactin on events during primary metamorphosis (Fig 2 and Table I)

The average time taken for thyroxin-treated larvae and controls to proceed from the pre-melanic stage through complete metamorphosis was 12.8 and 15.3 days, respectively. This time differential was largely accounted for by the accel-

TABLE I

Average time in hours from initial injection for larvae of Diemictylus to pass through stages of primary metamorphosis

Treatment	Control	Thyroxin	Prolactin-Group I	Prolactin-Group II
Number of animals	17	6	6	5
Pre-melanic to melanic	199.2 ± 15.6	208.8 ± 40.6	168 ± 36.4	
Melanic to gill loss	297.6 ± 18.3	273.6 ± 21.9	348.8 ± 19.3	364.8 ± 6.1
Gill loss to complete metamorphosis	367.2 ± 5.3	307.2 ± 6.0	—	—

erated rate of metamorphosis in thyroxin-treated animals as compared with controls from the time of gill reabsorption to their emergence on land which in the former occurred within a 24-hour period.

An event of major significance in these experiments was the failure of prolactin-treated larvae to complete metamorphosis despite the fact that their gills had been reabsorbed as much as two weeks before the termination of observations. In addition, they showed a lower rate of metamorphosis between melanic and gill loss stages, taking as long as 15 days to make the transition, as compared to an average of 72 hours for the controls and thyroxin group. The time taken for prolactin-treated animals to pass through the pre-melanic stage was slightly less than that

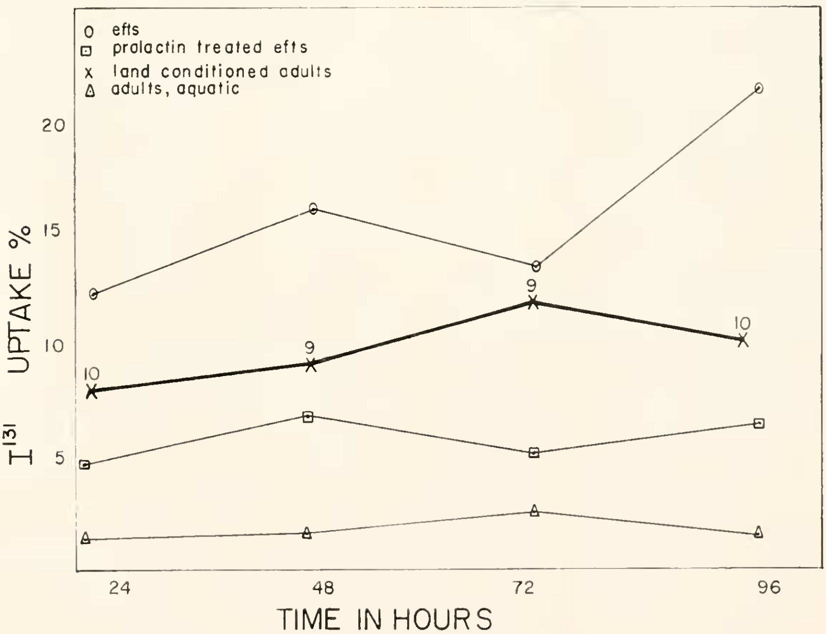

FIGURE 3. Per cent thyroid uptake of I^{131} by efts, prolactin-treated efts, normal adults and land-conditioned adults. The number of land-conditioned animals used in the determination at each point is indicated. Data on other phases are modified from Waterman and Grant (1961).

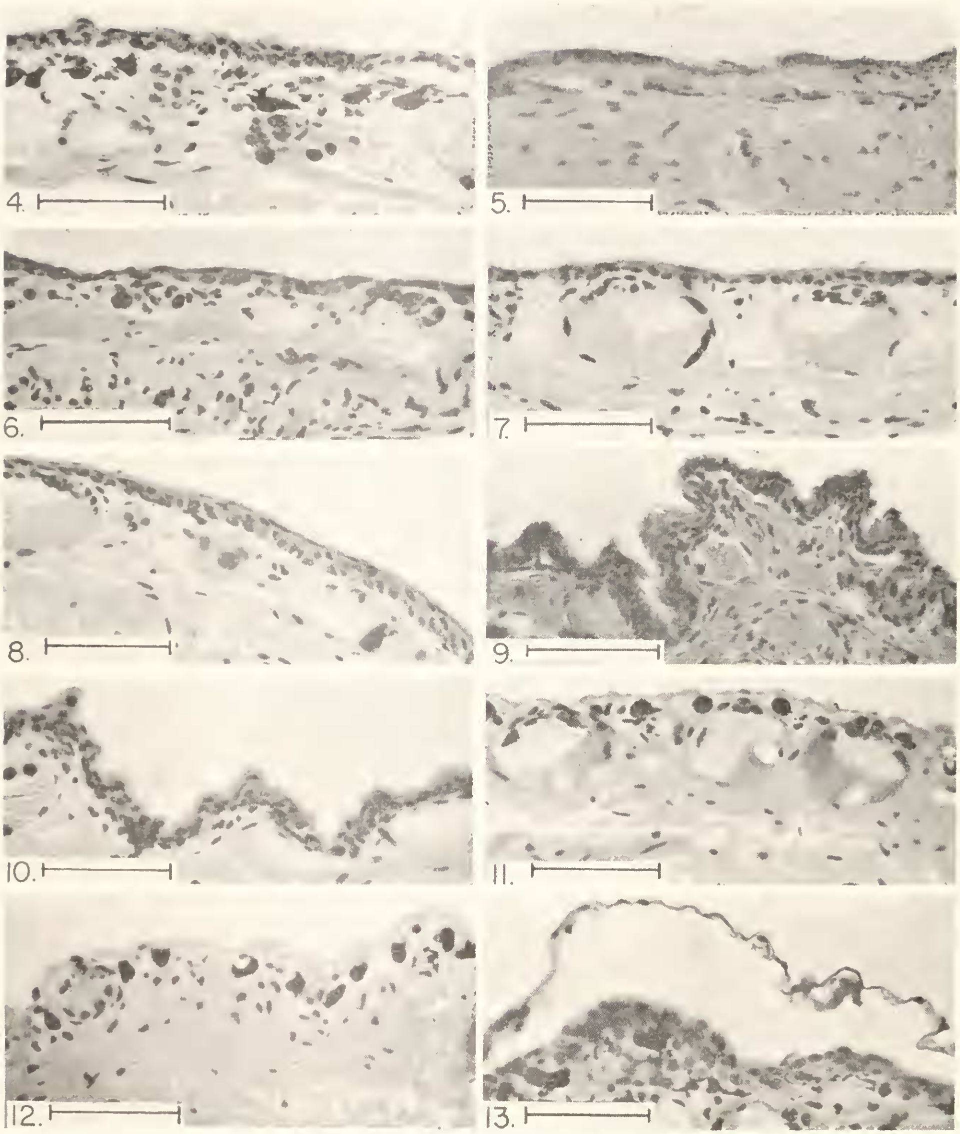

FIGURE 4. A section of skin taken from the back of an adult newt. The scale is 50 μ. Hematoxyln and eosin.

FIGURE 5. Back skin of adult animal (Group B) following two-week treatment with thyroxin, showing reduction of epidermis and glands. The scale is 50 μ. Hematoxylin and eosin.

FIGURE 6. Neck skin of a newt five days after cessation of thyroxin treatment, showing some epidermal thickening and enlargement of glands. The scale is 50 μ. Hematoxylin and eosin.

FIGURE 7. Skin from the neck of an adult newt after 45 days of prolactin injections. The epidermis is extremely smooth and glandular elements are well developed. The scale is 50 μ. Hematoxylin and eosin.

for the other groups. However, as there was no precise way of determining the actual age of pre-melanic animals at the time of their first injection, slight variations at this level cannot be considered particularly significant. It is interesting to note that larvae which remained aquatic after gill loss made appropriate behavioral adjustments by coming to the surface to obtain air in the manner described by Spurway and Haldane (1954) for adult newts, despite the fact that a favorable surface-to-volume ratio for cutaneous respiration undoubtedly existed.

Thyroid activity of terrestrial adults (Fig. 3)

The following daily values, expressed in per cent uptake of I^{131} by thyroids of adult animals maintained on land for a 10-day period, were recorded: 8.1 ± 1.9%, 24 hours; 9.3 ± 3.2%, 48 hours; 12.07 ± 0.9%, 72 hours; 10.2 ± 2.8%, 96 hours. In Figure 3 these values are compared with those previously taken for efts, normal adults and prolactin-treated efts by Waterman and Grant (1961). In the present study controls showed uptakes of 1.6 ± 1.1% and 0.6 ± 0.51% at 24 and 72 hours, which compare closely with those for normal adults in previous studies.

Histological and tissue culture studies

Because the results of tissue culture studies were best observed at the cellular level they are discussed here for comparative purposes with results obtained from histological studies of eft and adult skin.

A skin section from a normal adult is shown in Figure 4. Animals from group B showed an increase in glandular and molting activity several days after treatment with thyroxin. In two weeks, however, when these animals had migrated to land, their glands were either absent or greatly reduced and their epidermis was composed of a thin layer of cells covered with several layers of *stratum corneum* (Fig. 5). Figure 6 shows a return to the normal adult condition in animals from group B_2 five days after cessation of thyroxin injections. Glandular elements are present once more and there has been considerable epidermal proliferation. Thyroxin-treated animals underwent complete molts several days after initial injections but failed to do so after their emergence on land.

In general, prolactin treatment had similar results in both adults and efts. In these animals there was a marked and sustained increase in the size of mucous glands, and their epidermis became compact and smooth with a thin cornified layer.

FIGURE 8. Back skin of an eft four weeks after a single injection of prolactin (100 μg). The epidermis is smooth and compact. The scale is 50 μ. Hematoxylin and eosin.

FIGURE 9. Normal eft skin, showing folding of epidermis and presence of cornified tubercles. The scale is 50 μ. Hematoxylin and eosin.

FIGURE 10. Skin from adult newt of Group D kept in a terrestrial environment for six weeks. The rough, granular epidermis clearly resembles that of the eft (Fig. 9). The scale is 50 μ. Hematoxylin and eosin.

FIGURE 11. Section of adult skin maintained in organ culture for two weeks. Epithelium is a single, squamous layer and dermal atrophy is beginning. The scale is 50 μ. Hematoxylin and eosin.

FIGURE 12. Thyroxin-treated tissue culture of adult skin is shown at two weeks. There is a general disorganization of the dermis, melanophore migration, and a definite epidermis is lacking. The scale is 50 μ. Hematoxylin and eosin.

FIGURE 13. Prolactin-treated skin culture at two weeks showed a compact, proliferating epidermis which underwent occasional molts. The scale is 50 μ. Hematoxylin and eosin.

Figure 7 shows the skin of an adult of group C 40 to 45 days after initiation of treatment with prolactin and Figure 8 that of an eft 27 days after a single injection of 100 μg. prolactin. In the case of the eft there are major skin alterations involving the loss of skin folds and tubercles as compared to the granular skin of the normal eft (Fig. 9). It is interesting to note that in adults from group C there was a reversal of prolactin-induced changes by subsequent thyroxin injections, with the appearance of a more corrugated skin and reduced skin glands over a 15-day period. However, there is indication that previous prolactin treatment increased the latent period of thyroxin activity, if comparisons are made of this group and previously untreated group B animals which received thyroxin injections for the same period of 15 days.

Group D adults placed on land showed little skin change at first, but after several days there was a progressive increase in the size of their skin glands, followed by a thickening and folding of the epidermis. Eventually the skin became virtually indistinguishable from the normal eft condition, as can be seen by comparing skin from adults kept on land for 47 days (Fig. 10) with normal eft skin shown in Figure 9. Land-conditioned animals which returned to water assumed normal adult skin structure within 10 days. The molting activity of group D adults was similar to that of efts as the *stratum corneum* was constantly shed and replaced in patches rather than being sloughed as a unit.

It is clear that the skin of the adult animal can adjust readily to shifts in environment. Prolactin produced skin changes in the adult which paralleled those occurring in normal efts at the time of second metamorphosis. Thyroxin at the levels used in the present investigation, however, could only recapitulate some primary metamorphic changes in the adult, for although thyroid-treated animals responded by migrating to land, an eft-like epidermis never formed.

Adult skin sections in tissue culture were maintained as controls for a period of at least two weeks. At the end of five days there was considerable epidermal degeneration and many of the larger glands had been emptied of secretion. At 15 days (Fig. 11) dermal structure and glands were still reasonably well organized but tissue was covered with a single, loosely organized layer of squamous epithelium. In thyroxin-treated cultures glandular atrophy and degeneration of the epidermis occurred within a period of several days, and at two weeks (Fig. 12) the skin had lost most of its structural organization as compared with controls. Melanophores had migrated to the surface and a definitive epidermis was completely lacking. In contrast, prolactin-treated cultures were well maintained over a period of several weeks with little degeneration or distortion of cellular and tissue elements. The epidermis remained compact and underwent several molts during the course of the experiment. These were observed floating in wash solutions above the tissue fragments, as seen in Figure 13. No molts were observed in either the control or thyroxin cultures.

Discussion

The investigations described above indicate that profound changes occur in the behavior and skin structure of *Diemictylus* during the course of endocrine-induced primary and secondary metamorphosis. Thyroxin induces a land-drive in adults and acceleration of metamorphosis in larvae, while prolactin treatment initiates water-drive in efts and adults and inhibits metamorphosis of larvae. However, the causes of these events and such associated changes as skin morphology, molting and thyroid activity, etc., are complex.

It is generally accepted that the thyroid is involved with primary metamorphosis and molting mechanisms in urodeles. Clark and Kaltenbach (1961) and others have suggested that molting is directly influenced by thyroxin. The present studies indicate that, in addition, environment may play a significant role in determining both molting activity and skin conditions.

Thyroxin-injected adults showed a land-drive, but neither whole animals nor tissue cultures treated with thyroxin produced the heavy granular eft skin associated with the terrestrial habitat. Thyroxin had a deleterious effect on the integrity of the epidermis, which suggests either inhibition of growth of the *stratum germinativum*, or destruction of basement membranes, or both. Although some initial stimulation of molting did occur, it did not resemble that of the eft. Normal larvae in primary metamorphosis and adults kept on land, however, did develop eft-like skins. A possible interpretation is that assumption of the terrestrial environment itself stimulates the endogenous release of factors, including thyroxin, which control and maintain the eft condition after the organism metamorphoses from the larval state under the primary influence of thyroxin. The failure of thyroxin to maintain a viable skin in the present experiments is supported by the collagenolytic effect of thyroxin on basement membranes reported by Gross (1964).

Thyroid activity of terrestrially maintained adults, as indicated by per cent uptake of I^{131}, approaches the high values shown for efts by Waterman and Grant (1961) in contrast to the low uptakes reported for aquatic adults and prolactin-treated efts. Even if the limitations of the technique are considered, it is safe to conclude that increased thyroid activity is associated with events during and following primary metamorphosis and that this activity is decreased during secondary metamorphosis. Despite considerable controversy (Lewis and Frieden, 1959; Barch, 1953) about the effect of thyroxin on metabolic processes in amphibians, the above results suggest that increased thyroid activity may be necessary to meet the greater metabolic demands of the terrestrial organism.

The immediate effects of prolactin treatment on newts appear to be considerably more direct than those following thyroxin administration. With reasonable certainty prolactin can be considered to be the water-drive principle in *Diemictylus*. It appears to maintain the integrity of the characteristic type of epidermis formed during normal second metamorphosis in the intact animal and in tissue culture. For example, larvae treated with prolactin not only failed to undergo primary metamorphosis but assumed the characteristic skin structure of aquatic adults. The effects of prolactin on tissue growth and maintenance noted above are supported by the work of Chadwick and Jackson (1948), Berman *et al.* (1964), and by Niwelinski (1958), who found that prolactin stimulated differentiation in forelimb regenerates of *Triturus alpestris*. The relationship of prolactin activity to molting, however, remains obscure, although Grant and Grant (1958) reported partial molting after prolactin injections in hypophysectomized efts. Some stimulation of molting by prolactin is consistent with the view that epidermal proliferation is a distinct factor of the molting mechanism (Chadwick and Jackson, 1948).

The evidence assembled to date indicates that prolactin and thyroxin have certain antithetical properties with respect to their influence on metamorphic events in urodeles. Thyroid activity is directed by the hypophysial release of TSH under neurosecretory stimulation from the hypothalamus (Etkin, 1938, 1964; Etkin and Lehrer, 1960; Etkin and Sussman, 1961). Hypothalamic activity in turn may inhibit prolactin secretion (Masur, 1962). While major shifts in the levels of either thyroxin or prolactin may help produce the adjustments to environment associated with metamorphosis, undoubtedly a delicate balance exists between those factors which help to maintain the normal integument of both the aquatic and terrestrial forms during and after metamorphic events. This is apparently true in the case of anuran molting in which environmental and adrenocortical factors may be involved as well (Jorgensen and Larsen, 1960, 1961; Stefano and Donoso, 1964). Prolactin in general appears to have a more complete influence on conditioning independent physiological adjustment to environment than thyroxin, for whereas adults and even gill-bearing larvae (Noble, 1929) can be kept in a moist, terrestrial environment, efts can only be maintained in water after prolactin administration.

The possible evolution of prolactin and thyroxin activity paralleling the course of vertebrate evolution is suggested by these studies. The thyroid hormone and changes in response to its activity undoubtedly conditioned the assumption of terrestrial modes of life in Amphibia, whereas prolactin-like principles may have been initially involved with osmoregulatory forces operating upon the integument of organisms in fresh-water habitats and later with the water-drive responses associated with second metamorphosis and annual spawning activity. Therefore, one would expect a balanced thyroxin-prolactin system effecting terrestrial and aquatic adaptation to be most clearly defined in the more generalized urodele amphibians which indeed appears to be the case. Nicoll and Bern (1964) failed to demonstrate the presence of prolactin in organ cultures of teleost and chondrichthyian pituitary tissue with pigeon-crop assay. Grant and Pickford (1959) and Grant (1961), however, obtained positive water-drive response in efts treated with teleost, shark and skate pituitary brei. This suggests the possibility of either a range in the structure and activity of prolactin-like substances throughout the vertebrates, or progressive evolution in vertebrates of a parent "prolactin molecular entity," as suggested by Nicoll and Bern and Berman *et al.* (1964). One of the earliest activities of prolactin (or prolactin-like principles) may have been involved with maintaining osmoregulatory homeostasis in fresh-water organisms through its effect on integumentary systems. This suggestion is supported by the fact that mammalian prolactin affects fresh-water survival in *Fundulus* and in *Xiphophorus,* as shown by Pickford and Phillips (1959) and Schreibman and Kallman (1964) and the increased water-drive activity in pituitaries of *Bufo americana* and *B. fowleri* taken from ponds during the spawning season (Grant, unpublished). This homeostatic activity which was present in the earliest fresh-water vertebrates may be directly affected by the "water-drive" principle which is present throughout the vertebrate series. On the other hand, the prolactin growth factor which is associated with pigeon-crop-stimulating activities, as indicated by Berman *et al.* (1964), may have originated more recently at the time of tetrapod evolution in the Devonian. The taxic response shown by prolactin-treated efts in locating water (Grant, 1964) indicates that the water-drive principle has a direct effect on behavior *per se* in addition to its action on the integument. Such activity would, of course, have been essential to the first terrestrial vertebrates.

We wish to express our gratitude to Dr. C. H. Li and the Endocrine Study Section of the NIH, who supplied us with the several prolactin preparations; to Dr. G. Lawrence Vankin for his help with photomicrography; to Mr. Bruce MacDougal, who first suggested the land-migrating activity of thyroxin, and to Mrs. Jacqueline High for technical assistance.

Summary

1. After larval metamorphosis the eft (terrestrial) phase of *Diemictylus viridescens* can be induced to undergo second metamorphosis to the aquatic, adult phase with mammalian prolactin. In the present investigations adults receiving thyroxin (10 to 20 μg. every other day) showed a partial reversal to second metamorphosis by migrating to land. However, the thin, cornified epidermis did not approximate the granular and compact eft-like epidermis seen in adults which were maintained without treatment in a terrestrial environment.

2. Ovine prolactin (NIH) produced water migration in efts and inhibited primary metamorphosis in larvae, while maintaining the smooth, compact epidermis characteristic of the normal adult.

3. Reaction of adult skin fragments in tissue culture receiving either 0.2 mg./ml. thyroxin or 1 mg./ml. prolactin paralleled the above results: after several days thyroxin-treated skin had lost most of its structural organization as compared with controls while prolactin cultures were well maintained, with a compact epidermis which even molted on several occasions.

4. Iodine-131 uptake of land-maintained adults showed values ($12.07 \pm 0.9\%$) which approached those for efts, as compared to the low values for normal adults and prolactin treated efts.

5. The evolution of a prolactin-thyroxin axis during the course of vertebrate evolution is suggested. Thyroxin may have affected adaptation to terrestrial environments and prolactin adaptation to the fresh-water habitat. Although the early activity of prolactin may be associated with the water-drive principle, it is understood that changes in tissue response or in the structure of prolactin have greatly extended the range of action for prolactin (prolactin-like principles) in higher vertebrates.

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